BEST AVAILABLE COPY

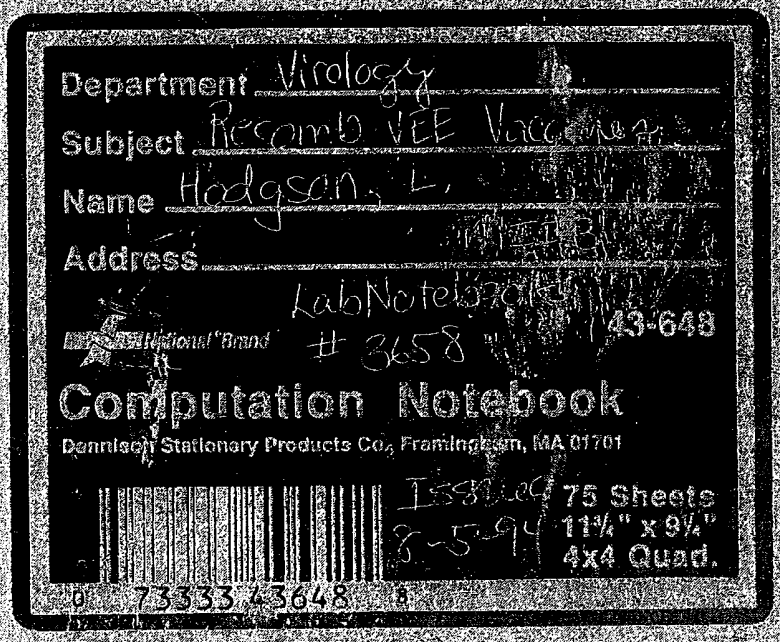
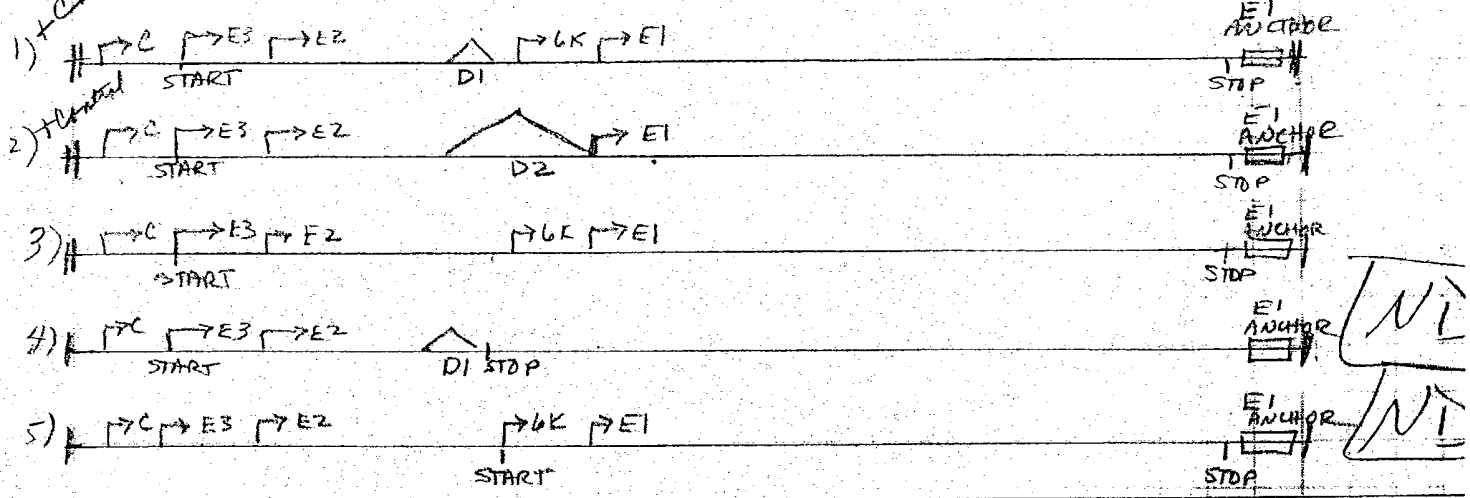


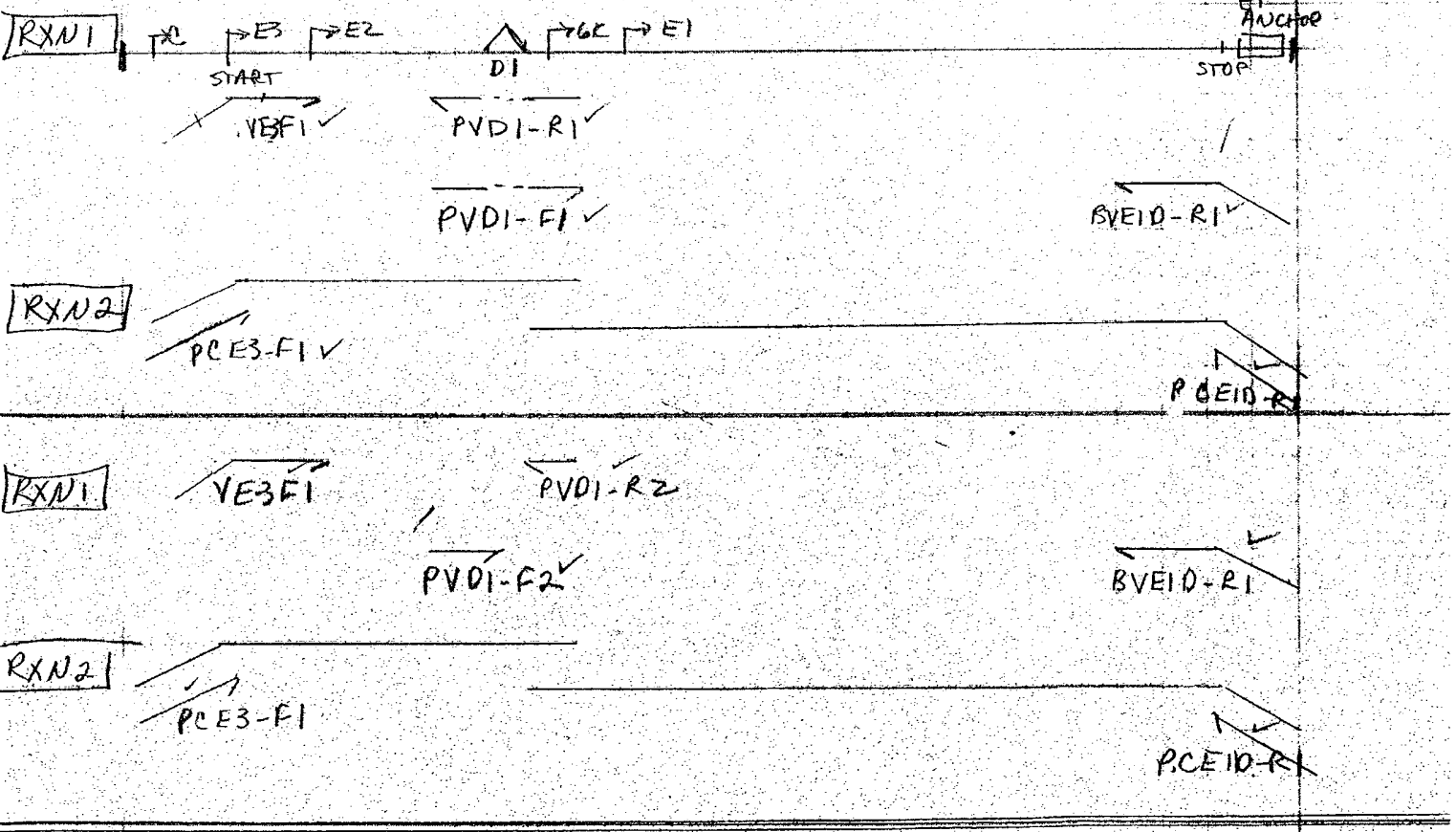
EXHIBIT U

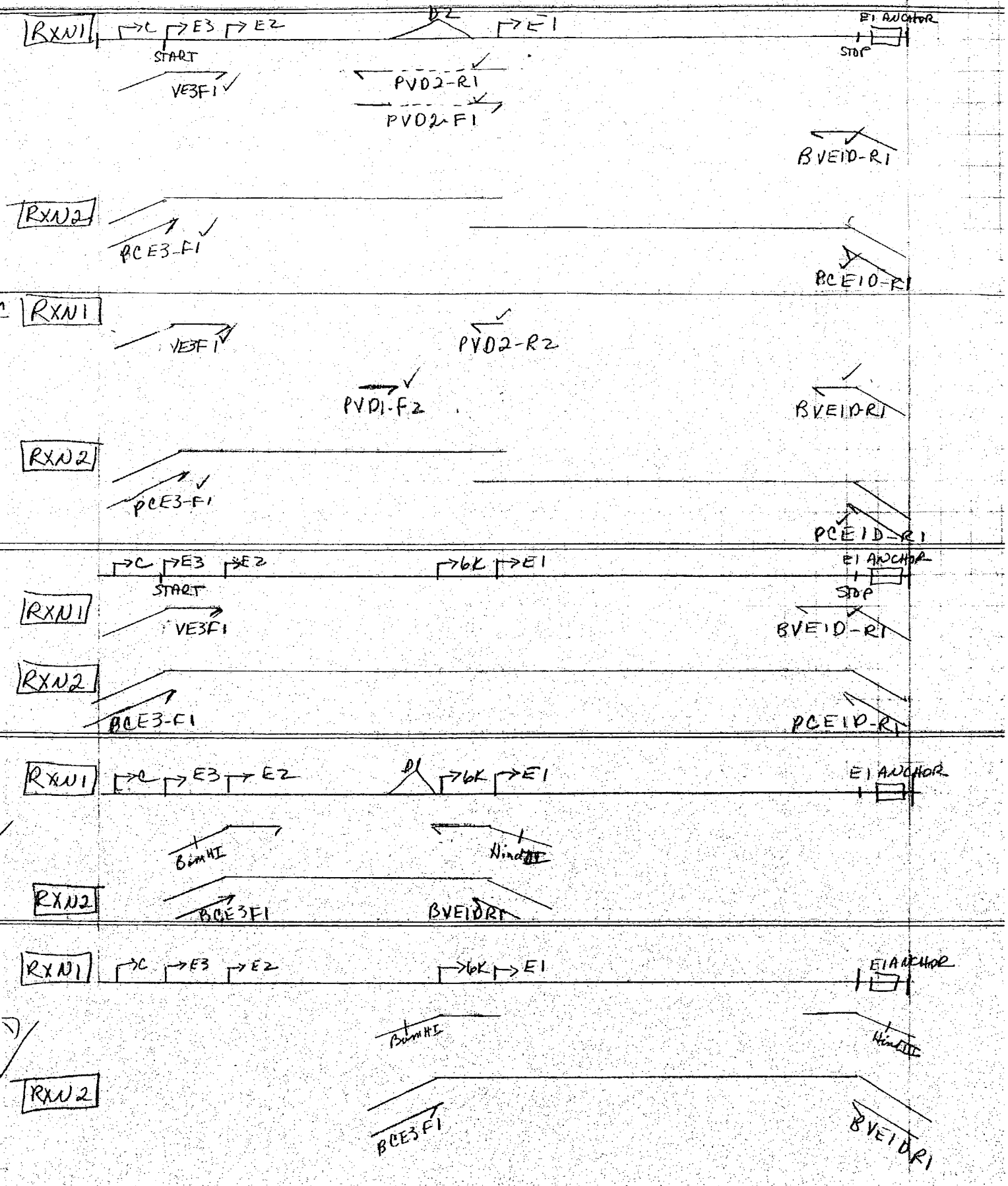
Primer design for the PCR amplification of E2 anchor deletion (D-1) and E2 + 6K deletion (D-2) for expression of proteins in baculoviruses. (pBB[illegible] - BamH1 + Hind III sites)

Plus

1) E3/E2 w/o E2 anchor (D1)
2) 6K/E1 w/o E1 anchor.






RXN1
C
E3
E2
D2
E1
START
E1 ANCHOR
STOP
VE3F1
PVD2-R1
PVD2-F1
BVE1D-R1
RXN2
PCE3-F1
PCE1D-R1
RXN1
VE3F1
PVD2-R2
PVD1-F2
BVE1D-R1
RXN2
PCE3-F1
PCE1D-R1
RXN1
C
E3
E2
6K
E1
E1 ANCHOR
START
STOP
VE3F1
BVE1D-R1
RXN2
BCE3-F1
PCE1D-R1
RXN1
C
E3
E2
D1
6K
E1
E1 ANCHOR
BamHI
NindIII
RXN2
BCE3F1
BVE1DR1
RXN1
C
E3
E2
6K
E1
E1 ANCHOR
BamHI
HindIII
RXN2
BCE3F1
BVE1DR1

Spacing my own primers

Primary PCR

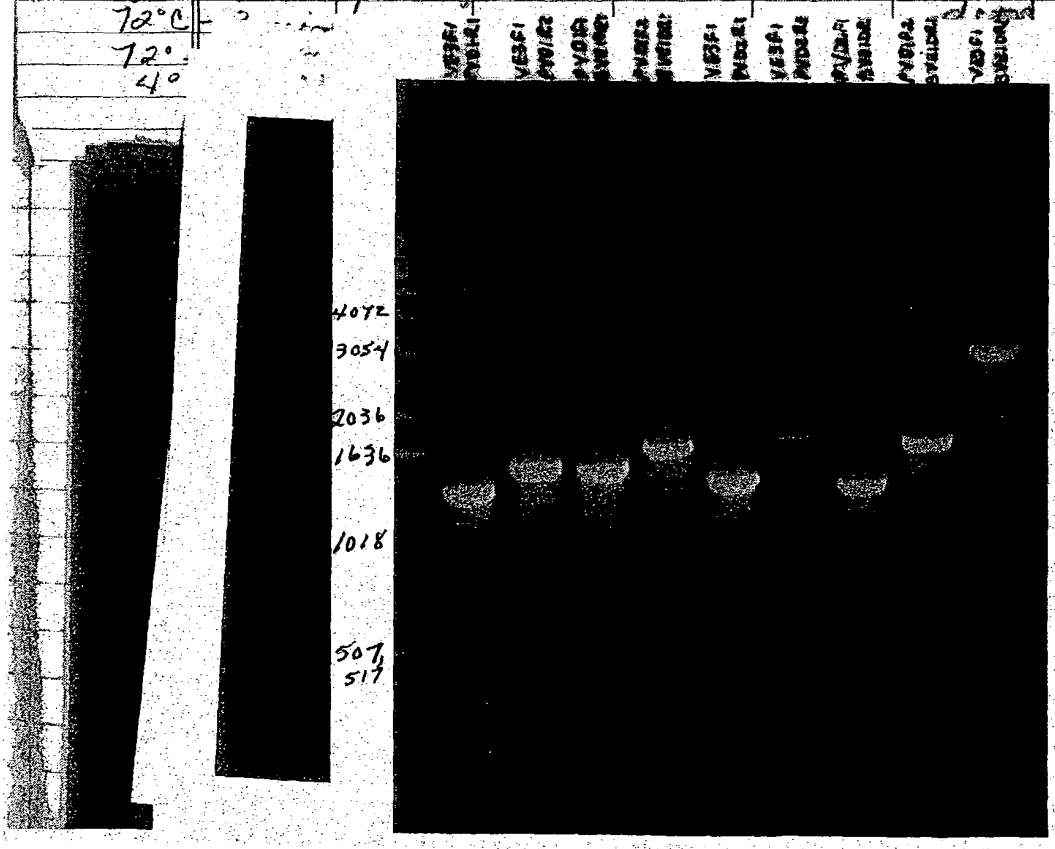
VEE CP D1, D2 with pBRIII

D1 = 165bp deletion

DNA TEMPLATE TUBE #	λC	1	2	3	4	5	6	7	8	9
				20WAT II - MAXIPREP 1ffEB94						
FORWARD PR		VE3F1	VE3F1	PVD1-F1	PVD1-F2	VE3F1	VE3F1	PVD2-F1	PVD1-F2	VE3F
REVERSE PR		PVD1-R1	PVD1-R2	BVE1D-R1	BVE1D-R1	PVD2-R1	PVD2-R2	BVE1D-R1	BVE1D-R1	BVE1
EXPECTED LENGTH		8358-9648 +20 1310	8358-9833 1475	1437	1602					
H_2O	10.5	77.7	78.3	80.5	78.7	77.5	78.2	78.3	78.5	77.
DNA TEMP.	1									
FORWARD PR	5	1.9	1.9	0.6	0.4	1.9	1.9	0.8	0.6	1.9
REVERSE PR	5	0.9	0.3	1.4	1.4	1.1	0.4	1.4	1.4	1.4
10X RXN BUFF.	10									
dNTPs	2nd lea									
	Vortex	microfuge								
Taqpol 0.5		22.3	21.7	19.5	21.3	22.5	21.7	21.7	21.5	22.5

960d program
95°C - 2 min
94°C - 30 sec
55°C - 1 min
72°C - 2 min
72° -
4° -
} 35 cycles

SUPERMIX
20WAT II: 1 x 10 = 10
10X RXN BUFFER: 10 x 10 = 100
dATP 2 x 10 = 20
2 x 10 = 20
2 x 10 = 20
2 x 10 = 20
19 rxns



T-162's

14 (D2)

Have SP1, SP2, SP3, SP4, SP5, SP6, SP7 constructs + D1 = PCR# 62
2 new deletion constructs D0, D2 (P1) to amplify - D0 = PCR# 4, D2 = PCR#
Have 2.2 x 10^6 cell/mL (~140 mL) = 308 x 10^6 cells total
T150's T162 cm^2 = 1.8 x 10^7 cells/flask - Enough for ~15 T162's
Set up 2 - T150's/construct SP1 - 7 tk + βgal + uninfected
minimum Virus Volume = 4 mL
Incubate in final volume = 20 mL
Add 8.8 mL of cells (18 mL media) each of 16 - T162's
Allow cells to adhere for 1H @ RT
(39.4 mL of cells + 166.6 mL of SF900

Have 2 flasks
GL = 2.2 x 10^6 cells/mL ~140 mL
Kristen = 2.9 x 10^6 cells/mL ~140 mL
Set up 21 T150's @ 1.8 x 10^7 cells flask = 378 x 10^6 cells total
Use 140 mL of GL = 308 x 10^6 cells
24 mL of Kristen = 69.6 x 10^6 cells
377.6 x 10^6 cells in 164 mL + 21 mL of

Titers: SP1: 4.4 x 10^8
2 5 x 10^7
3 2.3 x 10^8
4 8 x 10^7
5 1.6 x 10^8
6 1.4 x 10^8
7 1.8 x 10^8

mL of virus = (10 x 1.8 x 10^7) / (4.4 x 10^8) = (18 x 10^7) / (4.4 x 10^8) = 4.09 x 10^-1 = 0.4
= (18 x 10^7) / (5 x 10^7) = 3.6 mL
= (18 x 10^7) / (2.3 x 10^8) = 7.8 mL
= (18 x 10^7) / (8 x 10^7) = 2.25 mL
= (18 x 10^7) / (1.6 x 10^8) = 11.25 = 1.1 mL
= (18 x 10^7) / (1.4 x 10^8) = 12.86 = 1.3 mL
= (18 x 10^7) / (1.8 x 10^8) = 10 x 10^-1 = 1 mL

mL of virus = MOI x # of cells / titer
βgal = 1 x 10^8
(18 x 10^7) / (1 x 10^8) = 1.8

	VIRUS STOCK	mL of TMN-FH
SP1:	0.4	7.6
2	3.6 2.5	4.4
3	7.8 6	0.2
4	2.25	5.75
5	1.1	6.9
6	1.3	6.7
7	1	7
βgal	1.8	6.2
PCR# 4	0.2	7
PCR# 8	1	7
PCR# 62	1	7
63	1	7

After 1H, add 16 mL of Complete TMN to each flask = Make up 400 mL.